


VERIFICATION OF TRANSLATION

I, Takayoshi Hirose residing at Takahashi Bldg. Kita-sangokan, 13-3, Nishitenma 5-chome, Kita-ku, Osaka-shi, Osaka 530-0047 Japan, hereby declare that:

1. I read and understand the Japanese and English languages.
2. I translated Japanese Patent Application No. 2003-374944 from Japanese into English.
3. The annexed document is, to the best of my knowledge and belief, an accurate translation of Japanese Patent Application No. 2003-374944.

This 15th day of March 2011  
Osaka, Japan

  
Takayoshi Hirose

PATENT OFFICE  
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: November 4, 2003  
Application Number: Patent Application No. 2003-374944  
[JP2003-374944]  
Applicant(s): Shuji Miyagawa.  
The Animal Engineering Research Institute

December 3, 2004

Commissioner,  
Japan Patent Office

Hiroshi Ogawa (seal)

Certification No. 2004-3110709

[Name of the Document]	Application for Patent
[Agent's File Reference]	03YNH84
[Destination]	Commissioner, Patent Office
[International Patent Classification]	C12N 15/09 A61K 48/00
[Inventor]	
[Domicile or Residence]	12, Tsuchimachi 3-chome, Ashiya-shi, Hyogo
[Name]	Shuji Miyagawa
[Inventor]	
[Domicile or Residence]	5-5-43, Minami-kasugaoka, Ibaraki-shi, Osaka
[Name]	Katsuyoshi Matsunami
[Applicant]	
[Identification Number]	503198688
[Name]	Shuji Miyagawa
[Applicant]	
[Identification Number]	503200095
[Name]	The Animal Engineering Research Institute
[Agent]	
[Identification Number]	100085486
[Patent Attorney]	
[Name]	Takayoshi Hirose
[Indication of Fees]	
[Number of Advance Payment Ledger]	069339
[Amount of Payment]	¥21,000
[Attached Documents]	
[Name of Document]	Claims           1
[Name of Document]	Specification     1
[Name of Document]	Abstract          1
[General Power Number]	0310154

[General Power Number] 0310153

[Name of the document] Claims

[Claim 1]

An HLA-E chimeric molecule possessing the following amino acid sequence:

- (1) HLA-E chimeric molecule replacing all or part of  $\alpha 2$  domain of HLA-E molecule with all or part of  $\alpha 2$  domain of HLA-G1 molecule,
- (2) HLA-E chimeric molecule replacing, together with (1), signal peptide (SP) of HLA-E molecule with reformed SP partly reforming the SP of HLA-G1 molecule, or
- (3) HLA-E chimeric molecule replacing, together with (2), a part of amino acid sequence of  $\alpha 1$  domain and  $\alpha 2$  domain of HLA-E molecule, with a part of amino acid sequence of  $\alpha 1$  domain and  $\alpha 2$  domain of HLA-G1 molecule, respectively.

[Claim 2]

A base sequence for coding any HLA-E chimeric molecule of claim 1.

[Claim 3]

A nonhuman mammal cell or nonhuman mammal animal transformed by the base sequence of claim 2.

[Name of the document] Specification  
[Title of the invention] HLA-E Chimeric Molecule  
[Technical filed]

[0001]

The present invention relates to HLA-E chimeric molecule. More particularly, it relates to HLA-E chimeric molecules for providing nonhuman mammal cell with resistance to cytotoxicity by human NK cell, base sequences for coding the chimeric molecules, and nonhuman mammal cell and nonhuman mammal animal transformed by the base sequences.

[Background art]

[0002]

Organ transplantation is a very useful therapeutic method. Organ transplantation is classified into allotransplantation and xenotransplantation, which have their own merits and demerits. The human-to-human allotransplantation is an established method, but it is limited in the number of donors. Xenotransplantation from nonhuman mammal (for example, swine) to human is multiple in selection of grafts, but it is always accompanied by specific rejections such as hypoacute rejection (HAR) and acute vascular rejection (AVR).

To overcome such specific rejections of xenotransplantation, various methods have been proposed, such as a method of expressing human complement inhibitor in nonhuman mammals (for example, patent document 1), a method of decreasing Gal $\alpha$ 1,3Gal sequences (hereinafter referred to as  $\alpha$ -Gal antigen) of non-reducing terminals of sugar chains not existing in primates or humans but existing in nonhuman mammals (for example, patent document 2), and a method of knocking out genes of  $\alpha$ 1,3 galactosyl transferase responsible for generation of  $\alpha$ -Gal antigen (for example, non-patent documents 1 and 2).

[0003]

[Patent document 1] Japanese unexamined patent publication No. H11-239430

[Patent document 2] Japanese unexamined patent publication No. 2002-291372

[Non-patent document 1] Science 2002, 295, 1089

[Non-patent document 2] Nat. Biotechnol. 2002, 20, 251

[0004]

When a graft of nonhuman mammal is transplanted on human recipient, if the HAR can be overcome, the latter's antibody (anti- $\alpha$ -Gal antibody, etc.), complement, platelet, or natural killer (NK) cell may adhere to the former's cell, and the cell may be activated. The activated cell releases various cytokines, dissociates heparin, produces gaps against adjacent cells, exposes collagen of basement membrane, induces blood clotting reaction, closes blood vessels, and necrotizes grafts of nonhuman mammal (non-patent document 3). Such rejections are known as acute vascular rejections (AVR), and so far no method has been known to suppress efficiently the cytotoxicity of NK cell known as one of AVR factors.

[0005]

[Non-patent document 3] Xenotransplantation 1998, 5, 169

[0006]

NK cell adheres to target cells by way of two types of receptors. They are killer cell activating receptor for inducing cytotoxicity, and killer cell suppressing receptor for suppressing cytotoxicity by recognizing the own MHC class I molecule. When the signal from the former surpasses the signal from the latter, the target cell is necrotized, but when the signal from the latter surpasses the signal from the former, the target cell is not necrotized.

The human cell expresses HLA class I molecules (HLA-A, -B, -C, -E, -F, -G), and hence the human cell is not damaged by human NK cell. On the other hand, since the nonhuman mammal cell does not express HLA class I

molecules, it is damaged by human NK cell. Hence, a new method has been developed to avoid cell damage by human NK cell by transforming the nonhuman mammal cell by genes of human HLA-A, HLA-B, HLA-C, or HLA-G (patent document 3). However, HLA-A, HLA-B, HLA-C are polymorphic, having 175, 344, and 90 alleles respectively, and it is not practical to prepare nonhuman mammal cells applicable to each HLA.

On the other hand, HLA-E and HLA-G are not polymorphic, and it has been attempted to use HLA-E and HLA-G. As a result, it is relatively easy to express HLA-G on the surface of nonhuman mammal cell, but suppression of cytotoxicity by human NK cell is low, and to the contrary it is found not easy to express HLA-E on the surface of nonhuman mammal cell, but suppression of cytotoxicity by human NK cell is high (non-patent document 4).

For the purpose of increasing the HLA-E expressing amount on nonhuman mammal cell surface, it has been attempted to use base sequence for coding the HLA-E,  $\beta_2$  microglobulin and HLA-A2 leader peptide (Val-Met-Ala-Pro-Arg-Thr-Leu-Val-Leu), or base sequence for coding the leader peptide of HLA-G (Val-Met-Ala-Pro-Arg-Thr-Leu-Phe-Leu). However, the HLA-E expressing amount by these transformants and the suppression of cytotoxicity by NK cell were not sufficient (non-patent document 5).

[0007]

[Patent document 3] Japanese unexamined patent publication No. H11-510698

[Non-patent document 4] Transplantation Proceedings 2000, 32, 939

[Non-patent document 5] Transplantation 2002, 73, 1582

[Disclosure of the invention]

[Problems to be solved by the invention]

[0008]

The invention is devised to solve the problems of the prior art, and the inventors have been intensively studied about HLA-E chimeric molecule for



providing the nonhuman mammal cell with resistance to cytotoxicity by human NK cell, and prepared base sequences for coding

(1) HLA-E chimeric molecule replacing all or part of  $\alpha 2$  domain of HLA-E molecule with all or part of  $\alpha 2$  domain of HLA-G1 molecule,

(2) HLA-E chimeric molecule replacing, together with (1), signal peptide (SP) of HLA-E molecule with reformed SP partly reforming the SP of HLA-G1 molecule, or

(3) HLA-E chimeric molecule replacing, together with (2), part of amino acid sequence of  $\alpha 1$  domain and  $\alpha 2$  domain of HLA-E molecule, with part of amino acid sequence of  $\alpha 1$  domain and  $\alpha 2$  domain of HLA-G1 molecule, respectively, and

attempted to transform the nonhuman mammal cell by using them, and found that the expressing amount of HLA-E chimeric molecules is increased and that the resistance to cytotoxicity by human NK cell is increased, thereby completing the invention.

That is, the invention presents HLA-E chimeric molecules for providing nonhuman mammal cell with resistance to cytotoxicity by human NK cell, base sequence for coding them, and nonhuman mammal cells and nonhuman mammal animals transformed by the base sequences.

The reformed SP means a sequence of amino acid sequence of SP in which one or two or more amino acids are replaced or deleted, or one or more amino acids are added, for examples, the reformed SP includes SP (MAVMAPRTLVL LLSGALTLTETWA) prepared by reforming SP (MVVMAPRTLFL LLSGALTLTETWA) of HLA-G1 molecule.

Amino acid sequences and base sequences of HLA-E molecule and HLA-G1 molecule are described later.

[Means to solve the problems]

[0009]

To achieve the object, the invention presents HLA-E chimeric molecules

for providing nonhuman mammal cell with resistance to cytotoxicity by human NK cell, and base sequence for coding them, and more specific examples are chimeric molecules having the following properties and base sequences for coding them.

(1) HLA-E chimeric molecule (Amino acid sequence number 1) having reformed SP, being HLA-E chimeric molecule except that amino acid number 91-182 of  $\alpha 2$  domain of HLA-E molecule is replaced by amino acid number 91-182 of  $\alpha 2$  domain of HLA-G1 molecule and a base sequence (Base sequence number 1) for coding it;

(2) HLA-E chimeric molecule (Amino acid sequence number 2) having reformed SP, being HLA-E chimeric molecule except that amino acid number 137-182 of  $\alpha 2$  domain latter part of HLA-E molecule is replaced by amino acid number 137-182 of  $\alpha 2$  domain latter part of HLA-G1 molecule and a base sequence (Base sequence number 2) for coding it;

(3) HLA-E chimeric molecule (Amino acid sequence number 3) having reformed SP, being HLA-E chimeric molecule except that fore part amino acid number 137-150 of  $\alpha 2$  domain latter part of HLA-E molecule is replaced by fore part amino acid number 137-150 of  $\alpha 2$  domain latter part of HLA-G1 molecule and a base sequence (Base sequence number 3) for coding it;

(4) HLA-E chimeric molecule (Amino acid sequence number 4 or 5) having SP of HLA-E molecule or reformed SP, being HLA-E chimeric molecule except that amino acid number 147 of  $\alpha 2$  domain of HLA-E molecule is replaced by cysteine and a base sequence (Base sequence number 4 or 5) for coding it;

(5) HLA-E chimeric molecule (Amino acid sequence number 6 or 7) having SP of HLA-E molecule or reformed SP, being HLA-E chimeric molecule except that amino acid number 11 of  $\alpha 1$  domain of HLA-E molecule is replaced by alanine, and that amino acid number 147 of  $\alpha 2$  domain is replaced by cysteine and a base sequence (Base sequence number 6 or 7) for coding it; and

(6) Nonhuman mammal cell or nonhuman mammal animal provided with resistance to cytotoxicity by human NK cell, prepared and transformed by one base sequence for coding the HLA-E chimeric molecule in any one of (1) to (5) mentioned above.

[Effect of the invention]

[0010]

The HLA-E chimeric molecule of the invention can be expressed efficiently on nonhuman mammal cells, and the resistance to cytotoxicity by human NK cell can be applied to nonhuman mammal cells. Therefore, the HLA-E chimeric molecule of the invention can effectively prevent generation of cell damage or acute vascular rejection (AVR) by human NK cell caused at the time of xenotransplantation of cells, tissues and organs of nonhuman mammals on human recipients.

[Best mode for carrying out the invention]

[0011]

The human HLA class I molecule consists of signal peptide (SP),  $\alpha 1$  domain,  $\alpha 2$  domain,  $\alpha 3$  domain, and transmembrane (TM) domain, and further includes  $\beta_2$  microglobulin ( $\beta_{2m}$ ).

The human HLA class I molecule also presents antigenicity by sterically incorporating oligopeptide derived from signal peptide (SP) into the groove formed by  $\alpha 1$  domain and  $\alpha 2$  domain.

As mention above, it is relatively easy to transform the nonhuman mammal cell by using HLA-G gene, but a suppressing capacity of damage by human NK cell is low. To the contrary, a higher suppressing capacity of damage by human cell NK cell is obtained by transforming the nonhuman mammal cell by using HLA-E gene, but it is not easy to transform. Accordingly, in order to provide the nonhuman mammal cell with resistance to cytotoxicity by human NK cell, it has been attempted to prepare a base sequence for coding the HLA-E chimeric molecule by exchanging domains in

the HLA-E molecule and HLA-G1 molecule, transform the cell line of nonhuman mammals, and analyze increase or decrease of expressing intensity by FACS, by using anti-HLA antibody (B9.12.1 Cosmo Bio.). As a result, as shown in Examples below, it is found that the HLA-E expression amount of nonhuman mammal cell is increased when transformed by the base sequence for coding the following HLA-E chimeric molecules, and that the resistance to cytotoxicity by human NK cell is increased.

[0012]

(1) HLA-E chimeric molecule prepared by replacing SP (MVDGTL L L L L L S E A L T Q T W A) of HLA-E molecule with the reformed SP (M A V M A P R T L V L L L S G A L T L T E T W A; reformed SP used in Example below) which is prepared by reforming SP (M V V M A P R T L F L L L S G A L T L T E T W A) of HLA-G1 molecule, and replacing  $\alpha 2$  domain (amino acid number 91-182) of HLA-E molecule with  $\alpha 2$  domain (amino acid number 91-182) of HLA-G1 molecule,

(2) HLA-E chimeric molecule prepared by replacing SP of HLA-E molecule with the reformed SP mentioned above, and replacing latter part (amino acid number 137-182) of  $\alpha 2$  domain of HLA-E molecule with latter part (amino acid number 137-182) of  $\alpha 2$  domain of HLA-G1 molecule,

(3) HLA-E chimeric molecule prepared by replacing SP of HLA-E molecule with the reformed SP, and replacing fore part (amino acid number 137-150) of latter part of  $\alpha 2$  domain of HLA-E molecule with fore part (amino acid number 137-150) of latter part of  $\alpha 2$  domain of HLA-G1 molecule,

(4) HLA-E chimeric molecule prepared by replacing or not replacing SP of HLA-E molecule with the reformed SP, and replacing serine of amino acid number 147 of  $\alpha 2$  domain of HLA-E molecule with cysteine of amino acid number 147 of  $\alpha 2$  domain of HLA-G1 molecule, and

(5) HLA-E chimeric molecule prepared by replacing or not replacing SP of HLA-E molecule with the reformed SP, and replacing serine of amino acid

number 11 of  $\alpha 1$  domain of HLA-E molecule and serine of amino acid number 147 of  $\alpha 2$  domain of the same with respectively alanine of amino acid number 11 of  $\alpha 1$  of HLA-G1 molecule and cysteine of amino acid number 147 of  $\alpha 2$  of the same.

[0013]

By building up a transgene by using a base sequence for coding one of the HLA-E chimeric molecules of (1) to (5) mentioned above, and gene promoter (for example,  $\beta$ -actin promoter, pMCP promoter, etc.), and/or other expression adjusting sequence, and transforming the nonhuman mammal cell by using the transgenes, it is possible to prepare nonhuman mammal cell having resistance to cytotoxicity by human NK cell.

By injecting the transgene into a fertilized egg of nonhuman mammal by microinjection method, it is possible to prepare nonhuman transgenic mammals composed of cells, tissues and organs having resistance to cytotoxicity by human NK cell. In the invention, nonhuman transgenic mammals are not particularly specified as far as nonhuman, and examples include swine, mouse, rat, hamster, cow, horse, sheep, rabbit, dog and cat, and considering xenotransplantation, swine may be preferred as a donor.

Further, by applying the nuclear-transfer method using the nonhuman mammal cell having resistance to cytotoxicity by human NK cell as donor cells, it is possible to prepare nonhuman cloned mammals composed of cells, tissues and organs having resistance to cytotoxicity by human NK cell. These nonhuman transgenic mammals or nonhuman cloned mammals can be prepared by properly selecting from the known methods and conditions.

[0014]

Amino acid sequences of HLA-E are as follows;

Amino acid sequence of HLA-E

MVDGTL L L L L L SEALAL TQTWAGSHSLKYFHTSVSRPGRGEPRFISVGYYDDTQFVRFDNDAA SPRMV PRA  
PWMEQEGSEYWDRETRSARDTAQIFRVNLR TLRGYYNQSEAGSHTLQW MHGCELGPD RRFLRGYEQFAYD  
GKDYLT L NED LRSW TAVDTAAQISEQKSN DASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHV  
THHPISDHEATLR C W A L G F Y P A E I T L T W Q D G E G H T Q D T E L V E T R P A G D G T F Q K W A A V V P S G E E Q R Y T C  
H V Q H E G L P E P V T L R W K P A S Q P T I P I V G I I A G L V L L G S V V S G A V V A A V I W R K K S S G G K G G S Y S K A E W S D S A  
QGSESHSL\*

(SP)

MVDGTL L L L L L SEALAL TQTWA

( $\alpha 1$  domain)

GS H S L K Y F H T S V S R P G R G E P R F I S V G Y V D D T Q F V R F D N D A A S P R M V P R A  
P W M E Q E G S E Y W D R E T R S A R D T A Q I F R V N L R T L R G Y Y N Q S E A

( $\alpha 2$  domain)

G S H T L Q W M H G C E L G P D R R F L R G Y E Q F A Y D  
G K D Y L T L N E D L R S W T A V D T A A Q I S E Q K S N D A S E A E H Q R A Y L E D T C V E W L H K Y L E K G K E T L L H L

( $\alpha 3$  domain)

E P P K T H V  
T H H P I S D H E A T L R C W A L G F Y P A E I T L T W Q D G E G H T Q D T E L V E T R P A G D G T F Q K W A A V V P S G E E Q R Y T C  
H V Q H E G L P E P V T L R W

(Transmembrane domain)

K P A S Q P T I P I V G I I A G L V L L G S V V S G A V V A A V I W R K K S S G G K G G S Y S K A E W S D S A  
Q G S E S H S L \*

[0015]

Base sequence of HLA-E is as follows;

Base sequence of HLA-E

atggtagatggaaccctccttttactcctctcggaggccctggcccttaccagacctgg  
gcgggctcccactccttgaagtatttccacactccgtgtcccgcccgccgcggggag  
ccccgttcatctctgtgggctacgtggacgacaccagttcgtgcgcttcgacaacgac  
gccgcgagtccgaggatggtgccgcgggcccgtggatggagcaggagggtcagagtat  
tgggaccgggagacacggagcgcaggggacaccgcacagatttccgagtgaatctgcgg  
acgctgcgcgggctactacaatcagagcgaggccgggtctcacacccctgcagtggatgcat  
ggctgcgagctggggcccgacaggcgcttccctccgcgggtatgaacagttcgcttacgac  
ggcaaggattatctcaccctgaatgaggacctgcgctcctggaccgggtggacacggcg  
gctcagatctccgagcaaaagtcaaatgatgccctgaggcggagcaccagagagcctac  
ctggaagacacatgcgtggagtggctccacaaatacctggagaagggggaaggagagcgtg  
cttcacctggagccccaaagacacagctgactcaccaccccatctctgacatgaggcc  
acctgaggtgctggggccctgggcttctacccctgcggagatcacactgacctggcagcag  
gatggggaggggccataccaggacacggagctcgtggagaccaggcccgaggggatgga  
accttcagaagtgggcagctgtggtggtgcccttctggagaggagcagagatacacgtgc  
catgtgcagcatgaggggctacccgagcccgctacccctgagatggaagccggcttcccag  
cccacatccccatcgtgggcatcattgctggcctggttctccttggaatctgtggtctct  
ggagctgtggttgcctgtgtgatatggaggaagaagagctcaggtggaaaaggaggagc  
tactctaaggctgagtgagcgcacagtgccaggggctcgtgagctcacagcttgtaa

[0016]

Amino acid sequences of HLA-G1 are as follows;

Amino acid sequence of HLA-G1

MVVMAPRTLFLLLSGALTLTETWAGSHSMRYFSAAVSRPGRGEPRIAMGYVDDTQFVRFSDSACPRME  
PRAPWVEQEGPEYWEETRNKKAHAQTDRMNLQTLRGYYNQSEASHTLQWMIGCDLGSDGRLLRGYEQY  
AYDGKDYALNEDLRSWTAADTAAQISKRKCEAAANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPK  
THVTHHPVDFYEATLRCWALGFYPAEIILTWORDEDQTDQVELVETRPAGDGTFOKWAAVVPSGEEQR  
YTCHVQHEGLPEPLMLRWKQSSLPTIPIMGIVAGLVVLAAVVTGAAVAVALWRKKSSD\*

(SP)

MVVMAPRTLFLLLSGALTLTETWA

( $\alpha 1$  domain)

GSHSMRYFSAAVSRPGRGEPRIAMGYVDDTQFVRFSDSACPRME  
PRAPWVEQEGPEYWEETRNKKAHAQTDRMNLQTLRGYYNQSEA

( $\alpha 2$  domain)

SSHTLQWMI GCDLGSDGRLLRGYEQY  
AYDGKDYALNEDLRSWTAADTAAQISKRKCEAAANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRA

( $\alpha 3$  domain)

DPPK  
THVTHHPVDFYEATLRCWALGFYPAEIILTWORDEDQTDQVELVETRPAGDGTFOKWAAVVPSGEEQR  
YTCHVQHEGLPEPLMLRW

(Transmembrane domain)

KQSSLPTIPIMGIVAGLVVLAAVVTGAAVAVALWRKKSSD\*



[0017]

Base sequence of HLA-G1 is as follows;

Base sequence of HLA-G1

atgggtggtcatggcgccccgaacctcttccctgctactctcggggggccctgacctgacc  
gagacctgggcgggctccacacctatgaggtatttcagcgccgctgtcccggccggc  
cgcggggagccccgcttcacgccatgggctacgtggacgacacgcagtctgtcgggttc  
gacagcgactcggcgtgtccgaggatggagccgcgggcgcgtgggtggagcaggagggg  
ccagagtattgggaaggagagacgggaacaccaaggcccacgcacagactgacagaaatg  
aacctgcagacctgcgcggctactacaaccagagcggagccagttctcacacctccag  
tggatgatgggtgcgacctggggtccgacggtcgccctctccgcgggtatgaacagtat  
gcctacgatggcaaggattacctcgccctgaacgaggacctgcgctcctggaccgcagcg  
gacactcgggctcagatctccaagcgcaagtgtgaggcgccaaatgtggctgaacaaagg  
agagccctacctggagggcacgtgcgtggagtggctccacagataccggagaacgggaag  
gagatgctgcagcgcgcggaccccccaagacacacgtgacccaccacctgtctttgac  
tatgaggccacctgaggtgctgggcccctgggcttctacctgcggagatcatactgacc  
tggcagcgggatggggaggaccagaccaggacgtggagctcgtggagaccaggcctgca  
ggggatggaaccttcagaagtgggcagctgtggtggtgccctctggagaggagcagaga  
tacacgtgccatgtgcagcatgaggggctgccggagccccctatgctgagatggaagcag  
tcttccctgcccaccatccccatcatgggtatcgtgctggcctgggtgtctcttcagct  
gtagtcactggagctgcggtcgtgctgtgctgtggagaagaagagctcagattga

[0018]

The sequences of amino acid sequence number 1 are as follows;

Amino acid sequence number 1

(Reformed SP) :

MAYMAPRTLVL LLSGALTLT ETWA

( $\alpha$ 1 domain) :

GSHSLKYFHT SVSRPGRGEP RFISVGYVDD TQFVRFDNDA  
ASPRMVPRAP WMEQEGSEYW DRETRSARDT AQIFRVNLRT  
LRGYYNQSEA

( $\alpha$ 2 domain) :

SSHTLQWMIG CDLGSDGRILL RGYEQYAYDG KDYLALNEDL  
RSWTAADTAA QISKRKCEAA NVAEQRRAYL EGTCVEWLHR  
YLENGKEMLO RA

( $\alpha$ 3 domain) :

EPPKTHVTHH PISDHEATLR CWALGFYPAE ITLTWQQDGE  
GHTQDTELVE TRPAGDGTFQ KWAAVVPSG EEQRYTCHVQ  
HEGLPEPVTLRW

(Transmembrane domain) :

KPASQPTIPI VGIAGLVLL GSVVSGAVVA AVIWRKKSSG  
KKGGSYSKAE WSDSAQGES HSL\*

[0019]

The sequences of base sequence number 1 are as follows;

Base sequence number 1

(Reformed SP) :

atggcggtcatggcgccccgaacctcgctcgtctcctctctcggggccctgacctgacc  
gagacctgggcg

( $\alpha 1$  domain) :

ggctccactccttgaagtatttcacacttccgtgtcccgccccggccggggagccc  
cgcttcatctctgtgggctacgtggacgacacccagttcgtgcgttcgacaacgacgcc  
gcgagtcggaggtgggtcccgccggcgccgtggatggagcaggaggggtcagagtattgg  
gaccgggagacacggagcgccaggacacgcacagattttccgagtgaatctgcggagc  
ctgcgcggctactacaatcagagcgaggcc

( $\alpha 2$  domain) :

agtctcacaccctccagtggtgattggctgcgacctgggtccgacgtcgcctctc  
cgcggtatgaacagtatgcctacgatggcaaggattacctcgccctgaacgaggacctg  
cgctcctggaccgcagcgacactcggctcagatctccaagcgaagtgtgagcgcc  
aatgtgctgaacaaaggagagcctacctggagggcacgtgcgtggagtggctccaga  
tacctggagaacgggaaggagatgctgcagcgcgcg

( $\alpha 3$  domain) :

gagccccaaagacacacgtgactcaccacctctctgacctgaggccacctgagg  
tgtggggccctgggcttctacctcgcgagctcacactgaactggcagcaggatggggag  
ggccataccaggacacggagctcgtggagaccaggcctgcaggggatggaaccttcag  
aagtgggcagctgtggtggtgccttctggagaggagcagagatacacgtgccatgtcag  
catgaggggctaccggagcccgtaacctgagatgg

(Transmembrane domain) :

aagccggcttccagcccaccatccccatcgtgggcatcattgctggcctggttctcctt  
ggatctgtggttctggagctgtggttgcgtgctgtgataggaggaagaagactcaggt  
ggaaaggaggagctactctaaggctgagtgagcgacagtgccccgggtctgagtct  
cacagcttata

[0020]

The sequences of amino acid sequence number 2 are as follows;

Amino acid sequence number 2

(Reformed SP) :

MAVMAPRTL V LLSGALTLT ETWA

( $\alpha$ 1 domain) :

GSLSLK YFHT SVSRPGRGEP RFISVG YVDD TQFVRFDNDA  
ASPRMVPRAP WMEQEGSEYW DRETRSARDT AQIFRVNLRT  
LRGYYNQSEA

( $\alpha$ 2 domain) :

GSHTLQWMHG CELGPDRRFL RGYEQFAYDG KDYLTLNEDL  
RSWTAVD TAA QISKRKCEAA NVAEQRRAYL EGTCTVEWLHR  
YLENGKEM LQ RA

( $\alpha$ 3 domain) :

EPPKTHVTHH PISDHEATLR CWALGFYPAE ITLTWQQDGE  
GHTQDTELVE TRPAGDGTFQ KWAAVVPSG EEQRYTCHVQ  
HEGLPEPVT L RW

(Transmembrane domain) :

KPASQPTIPI VGIAGLVLL GSVVSGAVVA AVIWRKKSSG  
GKGGSYSKAE WSDSAQGSSES HSL\*

[0021]

The sequences of base sequence number 2 are as follows;

Base sequence number 2

(Reformed SP) :

atggcggtcatggcgcccgaaacctcgtcctgctactctcgggggcccctgacctgacc  
gagacctgggcg

( $\alpha 1$  domain) :

ggctccactccttgaagtattccacacttccgtgtcccggccggccggggagccc  
cgcttcatctctgtgggtacgtggacgacacccagttcgtgccttcgacaacgacggc  
gcgagtcggaggtggtgccggggcgccgtggatggagcaggaggsgtcagagtattgg  
gaccgggagacacggagcggcaggacaccgcacagattttccgagtgaaatctcgggacg  
ctgcgggctactacaatcagagcgagggc

( $\alpha 2$  domain) :

gggtctcacacctgcagtggatgcatggctgcgagctggggcccgacagggcgttctc  
cgcggtatgaacagttcgctacgacggcaaggattatctaccctgaatgaggacctg  
cgctcttggaccgcggtggacactgcgggtcagatctccaagcgcaagtgtgaggcgccaatg  
tggtctgaacaaaggagagcctacctggaggggcacgtgcgtggagtggtccacagatacct  
ggagaaacggggaaggagatgctgcagcgcgcg

( $\alpha 3$  domain) :

gagccccaagacacacgtgactcaccacccatctctgacatgaggccacctgagg  
tgctggggccctgggttctaccctgcggagctcacactgacctggcagcaggatggggag  
ggccataccaggacacggagctcgtggagaccaggcctgcaggggatggaaacctccag  
aagtgggcagctgtggtggtccttctggagaggagcagagatacacgtgccatgtgcag  
catgaggggctaccggagcccgctaccctgagatgg

(Transmembrane domain) :

aagcggcttcccagcccaccatcccacgtggggcatcattgctggcctggttctcctt  
ggatctgtggtctctggagctgtggttgctgtgataggaggaagaagagctcaggt  
ggaaaggaggggagctacttaaggctgagtgagcgacagtgcccaggggtctgagttc  
cacagcttgtaa

[0022]

The sequences of amino acid sequence number 3 are as follows;

Amino acid sequence number 3

(Reformed SP) :

MAVMAPRTLVL LLSGALTLT ETWA

( $\alpha$ 1 domain) :

GSHSLKYFHT SVSRPGRGEP RFISVGIVDD TQFVRFNDNA  
ASPRMVPRAP WMEQEGSEYW DRETRSARDA QIFRVNLRT  
LRGYYNQSEA

( $\alpha$ 2 domain) :

GSHTLQWMHG CELGPDRRFL RGYEQFAYDG KDYLTLNEDL  
RSWTAVDTAA QISKRKCEAA SEAEHQRAYL EDTCWEWLHK  
YLEKGKETLL HL

( $\alpha$ 3 domain) :

EPPKTHVTHH PISDHEATLR CWALGFYPAE ITLTWQQDGE  
GHTQDTELVE TRPAGDGTQ KWAAVVPSG EEQRYTCHVQ  
HEGLPEPVTLRW

(Transmembrane domain) :

KPASQPTIPI VGIAGLVLL GSVVSGAVVA AVIWRKKSSG  
GKGGSYSKAE WSDSAQGSES HSL\*

[0023]

The sequences of base sequence number 3 are as follows;

Base sequence number 3

(Reformed SP) :

atggcgggtcatggcgcccccgaaccctcgtcctgctctctcgggggcccctgacctgacc  
gagacctgggcg

( $\alpha 1$  domain) :

ggctcccaactccttgaagtatttcacacttcctgtccccggccggccgggggagccc  
cgcttcatctctgtgggctacgtggacgacaccagttcgtgcgcttcgacaacgacgcc  
gcgagtcaggatgggtgccggggcgccgttgatggagcaggaggggtcagagtattgg  
gaocggggagacacgggagcggcagggacacgcacagattttcagtgaaatctgcggacg  
ctgcgcggctactacaatcagagcgaggcc

( $\alpha 2$  domain) :

gggtctcacacctgcagtggatgcatggctgcgagctggggcccgacaggcgcttctc  
cgcgggtatgaacagttcgctacgacggcaaggattatctcacctgaatgaggacctg  
cgctcctggaccgggtggacactgcggctcagatctccaaagcgcaagtgtgagggggc  
tctgagggcgagcaccagagagcctacctggaagacacatgcgtggagtggctccacaat  
acctggagaagggaaggagacgctgctcacctg

( $\alpha 3$  domain) :

gagcccccgaagacacacgtgactcaccacccatctctgacctagggccacctgagg  
tgctgggcccctgggcttctacctcgggagctcacactgacctggcagcaggatggggag  
ggcatatccacaggacacggagctcgtggagaccaggccctgcaggggatggaaccttcag  
aagtgggcagctgtgtgtgtgcttctggagaggagcagagatacacctgccatgtgcag  
catgaggggctacccgagcccgtcacctgagatgg

(Transmembrane domain) :

aagccggcttccagcccaccatcccatcgtgggcatcattgctggcctggttctctt  
ggatctgtggtctctggagctgtgtgtgctgctgtgatatggaggaagaagagctcaggt  
ggaaaaggaggagctactctaaggctgagtgaggagcagtgccagggggtctgagctc  
cacagcttgtaa

[0024]

The sequences of amino acid sequence number 4 are as follows;

Amino acid sequence number 4

(SP of HLA-E) :

MVDGTL~~LL~~L SEALALTQ~~T~~W A

( $\alpha$ 1 domain) :

GSHSLKYFHT SVSRPGRGEP RFISVG~~Y~~VDD TQFVRF~~D~~NDA  
ASPRMVPRAP WMEQEGSEYW DRETRSARDT AQIFRVNLRT  
LRGYYNQSEA

( $\alpha$ 2 domain) :

GSHTLQWMHG CELGPDRRFL RGYEQFAYDG KDYLTLNEDL  
RSWTAVDTAA QISEQK~~C~~NDA SEAEHQRAYL EDTC~~V~~E~~W~~LHK  
YLEK~~G~~KETLL HL

( $\alpha$ 3 domain) :

EPPKTHVTHH PISDHEATLR CWALGFYPAE ITLTWQQDGE  
GHTQDTELVE TRPAGDGT~~F~~Q KWA~~A~~V~~V~~PSG EEQRYTCHVQ  
HEGLPEPVTL RW

(Transmembrane domain) :

KPASQPTIPI VGIIAGLVLL GSVVSGAVVA AVIWRKKSSG  
GKGGSSYSKAE WSDSAQGS~~E~~S HSL\*



[0025]

The sequences of base sequence number 4 are as follows;

Base sequence number 4

(SP of HLA-E) :

atggtagatgggaacctccttttactcctcggaggccctggcccttaccagacctgg  
gcg

( $\alpha 1$  domain) :

ggctccactccttgaagtatttcacacttcgtgtcccgcccgccggggagccc  
cgcttatctctgtgggtacgtggacgacaccagttcgtgcgttcgacaacgacgcc  
gcgagtcaggatgggtcccgccggcgccgtggatggagcaggaggggtcagagtattgg  
gaccgggagacacggagcgccaggacaccgcacagattttcggagtgaatctcgggagc  
ctgcgcggctactacaatcagagcgggcc

( $\alpha 2$  domain) :

gggtctcacacctgcagtggatgcatggctgcgagctggggcccacaggcgcttcctc  
cgcggggtatgaacagttcgctacgacggcaaggattatctcacctgaatgaggacctg  
cgctcctggaccgggtggacacggcggtcagatctccgagcaaaagtgtaatgatgcc  
tctgaggcggagcaccagagagccgtacctggaagacacatgcgtggagtggctccaaaa  
tacctggagaagggaaggagacgctgcttcacctg

( $\alpha 3$  domain) :

gagccccc aaagacacagctgactcaccacctatcttgaccatgaggccacctgagg  
tgctgggccctgggcttctacctcgcgagctcacactgacctggcagcagatggggag  
ggccataccaggacacggagctcgtggagaccaggcctgcaggggatggaacctccag  
aagtgggcagctgtggtggtgccttctggagaggagcagagatacagtgccatgtgcag  
catgaggggctaccggagcccgctacacctgagatgg

(Transmembrane domain) :

aagccggcttccagccaccatccccatcggtgggcatttgctggcctggttctcctt  
ggatctgtggtctctggagctgtggttgcgtgtgatatggaggaagaagagctcaggt  
ggaaaaggaggagctactctaaggctgagtgagcgacagtgccagggggtctgagtct  
cacagcttgtaa

[0026]

The sequences of amino acid sequence number 5 are as follows;

Amino acid sequence number 5

(Reformed SP) :

MAVMAPRTLVL LLSGALTIT ETWA

( $\alpha$ 1 domain) :

GSHSLKYFHT SVSRPGRGEP RFISVGYVDD TQFVRFDNDA  
ASPRMVPRAP WMEQEGSEYW DRETRSARDT AQIFRVNLR  
LRGYYNQSEA

( $\alpha$ 2 domain) :

GSHTLQWMHG CELGPDRRFL RGYEQFAYDG KDYLTLNEDL  
RSWTAVDTAA QISEQKCNDA SEAEHQRAYL EDTCVEWLHK  
YLEKGGKETLL HL

( $\alpha$ 3 domain) :

EPPKTHVTHH PISDHEATLR CWALGFYPAE ITLTWQQDGE  
GHTQDTELVE TRPAGDGTQ KWAAVVPSG EEQRYTCHVQ  
HEGLPEPVTL RW

(Transmembrane domain) :

KPASQPTIPI VGILAGLVLL GSVVSGAVVA AVIWRKKSSG  
GKGGSYSKAE WSDSAQGSES HSL\*

[0027]

The sequences of base sequence number 5 are as follows;

Base sequence number 5

(Reformed SP) :

atggcgggtcatggcgccccgaaccctcgtcctgcttactctcggggccctgaccctgacc  
gagacctgggcg

( $\alpha 1$  domain) :

ggctccactccttgaagtatttccacacttccgtgtcccgcccgccgcggggagccc  
cgcttcatctctgtgggtacgtggacgacacccagttcgtgcgttcgacaacgacgcc  
gcgagtcaggatgggtccgccccgcgcgtggatggagcaggaggggtcagagtattgg  
gaccgggagacacggagcgccaggacacgcacagattttccgagtgaatctgcggacg  
ctgcgcggctactacaatcagagcgaggcc

( $\alpha 2$  domain) :

gggtctcacaccctgcagtggtatgcatggctgcgagctggggcccgacaggcgcttctc  
cgcggttatgaacagttcgctacgacggcaaggattatctcacctgaatgaggacctg  
cgctctggaccggtggacacggcggtcagatctccgagcaaaagtgtaatgatgcc  
tctgagcgaggacaccagagagccgtacctggaagacacatgcgtggagtggtctccaaaa  
taacctggagaagggaaggagacgtgcttacctg

( $\alpha 3$  domain) :

gagccccaaagacacacgtgactcaccacccatctctgacctgaggccaccctgagg  
tgctggggccctgggcttctacctgcggagctcacactgacctggcagcaggatggggag  
ggccataccaggacacggagctcgtggagaccagcctgcaggggatggaaccttcag  
aagtgggcagctgtggtggtcctcttgagaggagcagagatacacgtgccatgtgcag  
catgaggggctacccgagcccgtaacctgagatgg

(Transmembrane domain) :

aagccggctcccagcccaccatcccacgtgggcatattgctggcctggttctcctt  
ggatctgtggtctctggagctgtggttgctgctgtgatatggaggaagaagagctcaggt  
ggaaaaggaggagctactctaaggctgagtgagcgacagtgccagggggtctgagttc  
cacagcttgtaa

[0028]

The sequences of amino acid sequence number 6 are as follows;

Amino acid sequence number 6

(SP of HLA-E) :

MVDGTL~~LL~~~~LL~~SEALALTQ~~T~~W A

( $\alpha$ 1 domain) :

GSHSLKYFHT ~~A~~VSRPGRGEP RFISVG~~Y~~VDD TQFVRFDNDA  
ASPRMVPRAP WMEQEGSEYW DRETRSARDT AQIFRVNLRT  
LRGYYNQSEA

( $\alpha$ 2 domain) :

GSHTLQWMHG CELGPDRRFL RGYEQFAYDG KDYLTLNEDL  
RSWTAVDTAA QISEQK~~C~~NDA SEAEHQRAYL EDTC~~V~~E~~W~~LHK  
YLEKGKETLL HL

( $\alpha$ 3 domain) :

EPPKTHVTHH PISDHEATLR CWALGFYPAE ITLTWQQDGE  
GHTQDTELVE TRPAGDGT~~F~~Q KWA~~A~~V~~V~~VPSG EEQRYTCHVQ  
HEGLPEPVTL RW

(Transmembrane domain) :

KPASQPTIPI VGIAGLVLL GSVVSGAVVA AVIWRKKSSG  
GKGGSYSKAE WSDSAQ~~G~~SES HSL\*

[0029]

The sequences of base sequence number 6 are as follows;

Base sequence number 6

(SP of HLA-E) :

atggtagatggaaccctccttttactcctctcggaggccctggcccttaccagacctgg  
gcg

( $\alpha 1$  domain) :

ggctccactccttgaagtattccacactgccgtgtcccggcccggccggggagccc  
cgtctactctctgtgggtacgtggacgacaccagttctgtcgtctcgacaacgacgcc  
cgagtcaggagatgggtccgcccggcgccgtggatggagcaggagggtcagagtattgg  
gacggggagacacggagcggcgggacaccgcacagattttccgagtgaatctcgggacg  
ctgcgggtactacaatcagagcgagggc

( $\alpha 2$  domain) :

gggtctcacaccctgcagtggatgcatggctgcgagctggggcccagaggcgcttcctc  
cgcggtatgaacagttcgctacgacggcaaggattatctaccctgaatgaggacctg  
cgctcctggaccggtggacacggcggtcagatctccgagcaaaagtgtaatgatgcc  
tctgaggcggagcaccagagagccgtacctggaagacacatgcgtggagtggtccacaaa  
tacctggagaaggggaaggagacgctgcttcacctg

( $\alpha 3$  domain) :

gagccccaaagacacacgtgactcaccacccatctctgacatgaggccaccctgagg  
tgctggggccctgggttctaccctgcggagctcacactgacctggcagcagatggggag  
ggcatataccagacacggagctcgtggagaccaggcctgcaggggatggaaccttccag  
aagtgggcagctgtggtgtgccttctggagaggagcagagatacacgtgccatgtgcag  
catgagggggtaccggagcccgtacacctgagatgg

(Transmembrane domain) :

aagccggcttcccagcccaccatcccacatcgtgggcatcattgtggcctggttctcctt  
ggatctgtggtctctggagctgtggttctgctgtgatatggaggaagaagactcaggt  
ggaaaaggaggagctactctaaggctgagtgaggcgacagtgcccagggggtctgagtct  
cacagcttgtaa

[0030]

The sequences of amino acid sequence number 7 are as follows;

Amino acid sequence number 7

(Reformed SP) :

MAVMAPRTLVL LLSGALTLT ETWA

( $\alpha$ 1 domain) :

GSHSLKYFHT AVSRPGRGEP RFISVGIVDD TQFVRFDNDA  
ASPRMVP RAP WMEQEGSEYW DRETRSARDT AQIFRVNLRT  
LRGYYNQSEA

( $\alpha$ 2 domain) :

GSHTLQWMHG CELGPDRRFL RGYEQFAYDG KDYLTLNEDL  
RSWTAVDTAA QISEQKCNDASEAEHQRAYL EDTCVELHLK  
YLEKGKETLL HL

( $\alpha$ 3 domain) :

EPPKTHVTHH PISDHEATLR CWALGFYPAE ITLTWQQDGE  
GHTQDTELVE TRPAGDGTFQ KWAAVVPSG EEQRYTCHVQ  
HEGLPEPVTL RW

(Transmembrane domain) :

KPASQPTIPI VGHLAGLVLL GSVVSGAVVA AVIWRKKSSG  
GKGGSYSKAE WSDSAQGSSES HSL\*

[0031]

The sequences of base sequence number 7 are as follows;

Base sequence number 7

(Reformed SP) :

atggcggctatggcgccccgaacctctgtctgctgctctctcgggggccccgacctgacc  
gagacctgggcg

( $\alpha 1$  domain) :

ggctccactccttgaagtatttccactgccgtgtccggccccggccgggggagccc  
cgcttcactctgtgggctacgtggacgacacccagttcgtgccttcgacaacgacgcc  
gcgagtcaggatgggtgccggggcgccgtggatggagcaggagggtcagagtattgg  
gaccgggagacacggagcgccaggacacgcacagattttccagtgaaatctcgggacg  
ctgcgcggctactacaatcagagcgaggcc

( $\alpha 2$  domain) :

gggtctcacacctgcagtggatgcatggctgcgagctggggcccgacagcgcttctc  
cgcggtatgaacagttcgctacgacggcaaggattatctcacctgaataggacctg  
cgctctggaccgggtggacacggcggtcagatctccgagcaaaagtgtaatgatgcc  
tctgaggcggagcaccagagagccgtacctggaagacacatgcgtggagtggctccacaaa  
tacctggagaagggggaaggagacgctgcttcacctg

( $\alpha 3$  domain) :

gagccccaaagacacacgtgactcaccacccatctctgacatgaggccacctgagg  
tgctggggccctgggcttctacctcgcgagctcacactgaactggcagcaggatggggag  
ggccataccaggacacggagctcgtggagaccaggcctgcaggggatggaaccttcag  
aagtgggcagctgtggtgcttctggagaggagcagagatacacgtgccatgtgcag  
catgaggggctaccggagcccgctacccctgagatgg

(Transmembrane domain) :

aagcggcttcccagcccaccatccccatcgtgggcacattgctggcctggttctcctt  
ggatctgtggttctggagctgtggttgctgtgatggaggaagaagagctcaggt  
ggaaaaggaggagctactctaaggctgagtgagcgacagtgcccaggggctgagctt  
cacagcttgtaa

[Example]

[0032]

The invention is more specifically described below by referring to Examples. However, it must be noted that the invention is not limited to these Examples alone.

#### Example 1

#### Expression of various HLA-E chimeric molecules in nonhuman mammal cell (1)

A base sequence for coding the amino acid sequence composed as shown in Table 1 was incorporated into an expression vector of pCXN ( $\beta$ -actin promoter of chicken, having enhancer of CMV). Each transgene was incorporated into CHO cell, and the relative value of expression amount was determined by FACS analysis by using anti-HLA antibody (Pan-Class I antibody, B9.12.1 Cosmo Bio). Results are shown in Table 1.



[0033]

Table 1. Expression of various HLA-E chimeric molecules in nonhuman mammal cell

Symbol	Molecule composition	Expression (relative amount)
(1) HLA-G1	HLA-G1 molecule	100
(2) HLA-E	HLA-E molecule	<1
(3) HLA-E(V)	Replaced SP of above (2) by Reformed SP	2
(4) E(V)-TM	Replaced TM of above (3) by TM of above (1)	2
(5) E(V)- $\alpha$ 3TM	Replaced $\alpha$ 3, TM of above (3) by $\alpha$ 3, TM of above (1)	<1
(6) E(V)- $\alpha$ 2	Replaced $\alpha$ 2 of above (3) by $\alpha$ 2 of above (1)	62
(7) E(V)- $\alpha$ 1 $\alpha$ 3TM	Replaced $\alpha$ 1, $\alpha$ 3, and TM of above (3) by $\alpha$ 1, $\alpha$ 3, and TM of above (1)	<1
(8) E(V)- $\alpha$ 1	Replaced $\alpha$ 1 of above (3) by $\alpha$ 1 of above (1)	<1
(9) E(V)- $\alpha$ 1-1	Replaced fore part of $\alpha$ 1 of above (3) by fore part of $\alpha$ 1 of above (1)	<1
(10) E(V)- $\alpha$ 1-2	Replaced latter part of $\alpha$ 1 of above (3) by latter part of $\alpha$ 1 of above (1)	<1
(11) E(V)- $\alpha$ 2-1	Replaced fore part of $\alpha$ 2 of above (3) by fore part of $\alpha$ 2 of above (1)	<1
(12) E(V)- $\alpha$ 2-2	Replaced latter part of $\alpha$ 2 of above (3) by latter part of $\alpha$ 2 of above (1)	29
(13) E(V)- $\alpha$ 2-2-1	Replaced fore part of latter part of $\alpha$ 2 of above (3) by fore part of latter part of $\alpha$ 2 of above (1)	26
(14) E(V)- $\alpha$ 2-2-2	Replaced hind part of latter part of $\alpha$ 2 of above (3) by hind part of latter part of $\alpha$ 2 of above (1)	4

(Note 1) Reformed SP: MAVMAPRTLVLSSGALTLTETWA

(Note 2) SP: signal peptide,  $\alpha$ 1:  $\alpha$ 1 domain,  $\alpha$ 2:  $\alpha$ 2 domain,  $\alpha$ 3:  $\alpha$ 3 domain, TM: transmembrane domain

[0034]

As known from the results in Table 1, it is confirmed that the HLA-E chimeric molecule is efficiently expressed on the CHO cell, by replacing the SP sequence of HLA-E molecule by the reformed SP (MAVMAPRTLVLSSGALTLTETWA) similar to SP of HLA-G1, and replacing  $\alpha$ 2 domain (amino acid number 91-182) of HLA-E molecule, latter part (amino

acid number 137-182) of  $\alpha 2$  domain of HLA-E molecule, or fore part (amino acid number 137-150) of latter part of  $\alpha 2$  domain of HLA-E molecule respectively by amino acid sequence corresponding to HLA-G1 molecule.

[0035]

## Example 2

### Expression of various HLA-E chimeric molecules in nonhuman mammal cell

(2)

A base sequence for coding the amino acid sequence composed as shown in Table 2 was incorporated into an expression vector of pCXN. Each transgene was incorporated into CHO cell, and FACS analysis was conducted by using anti-HLA antibody. Results are shown in Table 2.

[0036]

Table 2. Expression of various HLA-E chimeric molecules in nonhuman mammal cell

Symbol	Molecule composition	Peak of FACS
(1) Vector	Vector only	4.94
(2) HLA-E	HLA-E molecule	15.07
(3) HLA-E(V)	Replaced SP of above (2) by Reformed SP	36.84
(4) HLA-E(147)	Replaced serine of amino acid 147 of $\alpha 2$ domain of above (2) by cysteine	121.84
(5) HLA-E(V $\times$ 147)	Replaced SP of above (4) by Reformed SP	313.97

[0037]

As known from the results in Table 2, it is confirmed that the HLA-E chimeric molecule replacing serine of amino acid number 147 of  $\alpha 2$  domain of HLA-E molecule by cysteine of amino acid number 147 of  $\alpha 2$  domain of HLA-G1 molecule is efficiently expressed on the CHO cell, and it is also confirmed that the HLA-E chimeric molecule replacing SP sequence of HLA-E molecule by the reformed SP (MAVMAPRTLVL LLSGALTLTETWA) and replacing the amino acid sequence number 147 of  $\alpha 2$  domain of HLA-E molecule by cysteine is more efficiently expressed on the CHO cell. It is

further confirmed that the HLA-E chimeric molecule can be more efficiently expressed on the CHO cell by replacing the hydroxyl group ( $\cdot\text{OH}$ ) of  $\beta$ -position of serine by thiol group ( $\cdot\text{SH}$ ) of cysteine.

[0038]

### Example 3

#### Resistance of nonhuman mammal cell expressing HLA-E chimeric molecule to cytotoxicity by human NK cell

A base sequence for coding the amino acid sequence composed as shown in Table 3 was incorporated into an expression vector of pCXN. Each transgene was incorporated into swine endothelial cell (SEC), and stable cell lines were prepared. Human NK-like cells (YT) were applied to transformed SEC cells at a rate of 5:1 (37°C, 4 hours), and lactate dehydrogenase (LDH) released from SEC was detected as index, and the cytotoxicity by human NK cell was measured, and relative value of cytotoxicity was determined. Results are shown in Table 3.

[0039]

Table 3. Resistance of swine endothelia cells to cytotoxicity by human NK cell

Transformed cell	Description	Cytotoxicity (relative)
(1) SEC	No gene transduction operation	100 <sup>a</sup>
(2) Mock	Transduction of Mock gene	94 <sup>a</sup>
(3) HLA-E(V)	Transformation by base sequence of coding HLA-E chimeric molecule replacing SP of HLA-E molecule by reformed SP	85 <sup>b</sup>
(4) HLA-E(V, 147)	Transformation by base sequence of coding HLA-E chimeric molecule replacing SP of HLA-E molecule by reformed SP, and replacing amino acid 147 of $\alpha 2$ domain of HLA-E molecule by cysteine	35 <sup>c</sup>
(5) HLA-E(V, 11, 147)	Transformation by base sequence of coding HLA-E chimeric molecule replacing amino acid 11 of $\alpha 1$ domain by alanine, in addition to above (4)	21 <sup>d</sup>

(Note) a, b, c, d: There is significant difference among groups identified with different superscripts ( $P < 0.05$ ).

[0040]

As known from the results in Table 3, it is confirmed that cytotoxicity by human NK cell can be suppressed in swine endothelia cell transformed by base sequence for coding any one of HLA-E chimeric molecule replacing SP of HLA-E molecule by reformed SP; HLA-E chimeric molecule replacing SP of HLA-E molecule by reformed SP and replacing amino acid number 147 of  $\alpha 2$  domain of HLA-E molecule by cysteine; and HLA-E chimeric molecule replacing amino acid number 11 of  $\alpha 1$  domain by alanine in addition to the above.

[Name of the document]    Abstract

[Abstract]

[Subject]

The invention presents HLA-E chimeric molecules for providing nonhuman mammal cell with resistance to cytotoxicity by human NK cell, base sequences for coding the chimeric molecules, and nonhuman mammal cell and nonhuman mammal animal transformed by the base sequences.

[Means to solve the subject]

The HLA-E chimeric molecule of the invention is a peptide reforming all or part of signal peptide region,  $\alpha 1$  domain and/or  $\alpha 2$  domain of HLA-E, and the base sequence of the invention is a base sequence for coding the chimeric molecule. The transformant incorporating the base sequence of the invention is effective to express the HLA-E efficiently.